Marc Dann Declaration in Support of Motion for Class Certification

Exhibit 7

CURRICULUM VITAE

PERSONAL DETAILS

Name: Professor Charles Vyvyan HOWARD

Address: Centre for Molecular Biosciences
University of Ulster
Cromore Road
Coleraine, BT52 1SA

Date/Place of Birth: 22nd May 1946, Blackpool, UK

Nationality: British

RESEARCH METRICES

Google Scholar 21/06/2019: Publications 140: citations 8662; h-index 38; i10-index 86

FURTHER/HIGHER EDUCATION

Education: 1965-70 University of Liverpool: Faculty of Medicine. MB ChB

Qualifications: 1970 MB ChB, Liverpool
1971 Full General Medical Council Registration
1983 Ph D, Liverpool in developmental neurobiology
1995 MRCPath
1999 FRCPath

WORK EXPERIENCE

1970 - 1971 House Officer, Sefton General Hospital, Liverpool
1971 - 1975 Demonstrator in Anatomy, University of Liverpool
1975 - 1991 Lecturer in Anatomy, University of Liverpool

1991 – 2005	Senior Lecturer in Anatomy, University of Liverpool
2005 - 2014	Professor of Bioimaging, University of Ulster
2014-	Emeritus Professor of Bioimaging, University of Ulster

MEMBERSIP OF PROFESSIONAL BODIES

Fellow: - Royal College of Pathologists
- Royal Microscopical Society
- Collegium Ramazzini

Member: - British Society of Toxicological Pathologists

POSITIONS HELD ON PROFESSIONAL BODIES

1985-1992 General Editor, Journal of Microscopy
1991-1995 President International Society for Stereology
1996-1998 President Royal Microscopical Society
2003-2009 Member DEFRA Advisory Committee on Pesticides
2004-2006 Founding Editor of the journal 'Nanotoxicology'
2007-2009 President International Society of Doctors for the Environment

RESEARCH INTERESTS

Vyvyan Howard is a medically qualified pathologist with interests in low dose minimal change toxicology, particularly of the fetus and neonate. He has worked for many years in the field of developmental neurotoxicology, including the actions of pharmaceutical agents, pesticides and environmental pollutants. He is an expert on the quantification of toxicological change with the use of stereology. He has a long founded interest and expertise in imaging living processes through the use of microscopy. Recently he has held major grants for investigating the toxicology of nano-particles in biological systems. He has experience in regulatory toxicology, having served 6 years on the Advisory Committee on Pesticides in the UK. He has recently been appointed an expert in toxicology by the National Standards Authority of Ireland (NSAI) as a Member of the CEN/TC 436 Technical Committee addressing cabin air quality in commercial aircraft.

RESEARCH FUNDING WHILE AT University of Ulster

2006 – 2008 £250,000 STREP project 'Nanointeract' under EU Framework 6. Part of a € 3.3 million multicentre study on the fate and toxicity of engineered nanoparticles

2008 -2011 Organix Foundation £120,000 to investigate the effect of low dose exposure to hormone disrupting pesticides on fetal development

2008 – 2011 €420,000 EU FP7 grant NeuroNano to investigate the effect of nano-particles on the progression of Alzheimer's disease in a mouse model.

2010-2013 NC3R/MRC £360,000 to develop a 3D tissue model of human breast tissue for the testing of xenoestrogens.

Honours:

1989 - Awarded Royal Microscopical Society 150th Anniversary Gold Medal for services to microscopy.

1999 - Overall Winner, Caroline Walker Trust 11th Annual Awards for improving public health through food.

PUBLICATIONS

[1] Howard C.V., Scales L.E., & Lynch R. (1980).
'The numerical densities of alpha and gamma motoneurons in the trigeminal motor nucleus of the rat: A method of determining the separate numerical densities of two populations of anatomically similar cells.' *Mikroskopie (Wien)*, **37** (Suppl.), 220236

[2] Howard C.V. (1981).
'Experimental and theoretical evaluation of size distributions'. *Stereol.Iugosl*., **3**/Supplement 1, 7988

[3] Howard C.V, (1981).
'On the functional significance of the third moment of size distribution in biological systems.' *Stereol.Iugosl*., **3**/Supplement 1, 503510

[4] Maina J.N, Howard C.V. & Scales L.E. (1981).

'The determination of the length densities and size distributions of blood and air capillaries in the avian lung involving a log normal fitting procedure.' *Stereol.Iugosl*., **3**/Supplement 1, 673678

[5] Howard C.V., Lynch R., & Scales L. (1981).
'The numerical densities of alpha and gamma motoneurons in lamina IX of the cervical cord of the rat: a method of determining the separate numerical densities of two mixed populations of anatomically similar cells.' (1981). Eleventh International Congress of Anatomy: Advances in the Morphology of Cells and Tissues, 173-183. E.A. Vidrio & M. A. Galina (Eds.). Alan R. Liss, N.Y.

[6] Howard C.V. (1981).
'The separate numerical densities of alpha and gamma motor- neurones in the spinal cord of the rat.' (1981). *Sterol. Iugosl* **3**/suppl 1: 503510.

[7] Howard C.V., & Scales L. (1982).
'The concept of 'Neuromorphotaxis' based on a minimisation principle. A case for the critical analysis of biological variation.'. *Acta Stereol*.**1**: 241252.

[8] Lynch R. & Howard C.V. (1982).
'The use of horseradish peroxidase (HRP) in the stereological analysis of motor nuclei.' *Acta Stereol.* **1**: 253258

[9] Howard C.V. (1983)
PhD THESIS: 'Neuromicrosis: a process affecting the phylo- and ontogenetic development of the brain. An hypothesis based on stereological studies of neurone population characteristics.' The University of Liverpool. 1983.

[10] Scales L.E. & Howard C.V. (1983).
'Some empirical functions for use in the parametric modelling of stereological size distributions.' (1983). *Acta Stereol*, **2**: 187192

[11] Howard C.V., Scales L.E. & Allibone R. (1983)
'The relationship between Purkinje cell diameter and position within the cerebellar folia of the rat: a stereological analysis.' (1983). *Acta Stereol*.**2**:219222

[12] Howard C.V., & Eins S. (1984).
'Software solutions to problems in stereology.' (1984). *Acta Stereol*, **3**: 139158

[13] Howard C.V., Lynch R. & Woodhams P. (1985)

'Population characteristics of nerve cell bodies illustrated by the postnatal development of cerebellar granule cells in the rat.'. Quantitative Neuroanatomy in Transmitter Research. Eds. Agnati L.F. and Fuxe K. Macmillan, London. pp 4154

[14] Howard C.V., Reid S., Baddeley A. & Boyde A (1985).
'Unbiased estimation of particle density in the Tandem Scanning Reflected Light Microscope.' (1985). *J. Microsc*. **138**, 203 212

[15] Howard C.V. (1986).
'Rapid nuclear volume estimation in malignant melanoma using point-ampled intercepts in vertical sections.'. In: Quantitative Image Analysis in Cancer Cytology and Histology. Eds. Mary, J.Y. and Rigaut, J.P. Elsevier Science Publications B.V. pp. 245254

[16] Howard C.V. (1986).
'Stereological probes using 'optical sections' in scanning light microscopy.'. In: Science on Form. Eds. S. Ishizaka, Y. Kato, R. Takaki and J. Toriwaki. KTK Scientific Publishers, Tokyo. pp 137146

[17] Baddeley A., Howard C.V., Boyde A. & Reid S. (1987).
'Three-dimensional analysis of the spatial distribution of the particle, using Tandem Scanning Reflected Light Microscopy.' (1987). *Acta Stereol*, **6**: 87100

[18] Howard C.V. (1988).
'Unbiased measurements in electron microscopy.'. *Inst. Phys. Conf. Ser*. **93**, 4957.

[19] Evans S.M. & Howard C.V. (1989).
'A simplification of the step method for measuring mean section thickness.'. *J. Microscopy*. **154**: 289293

[20] Moss M.C., Browne M.A., Howard C.V. & Joyner D.(1989).
'An interactive image analysis system for measuring mean particle volume.' . *J. Microscopy*. **156**: 7990

[21] Braendgaard H., Evans S.M., Howard C.V. & Gundersen H.J.G.(1990).
'The total numbers of neurons in human neocortex unbiasedly estimated using optical disectors.'. *J. Microscopy*.**157**: 285304

[22] Howard C.V., Rassoli M.B. & McKerr G. (1990).
'Correlating structure with function in developmental neurology using stereological and confocal microscopical techniques.'. Science on Form. Ed. S. Ishizaka. KTK Scientific Publishers, Tokyo. pp 3137.

[23] Cogswell C.J., Sheppard C.J., Moss M.C. & Howard C.V. (1990).

'A method for evaluating microscope objectives to optimize performance for confocal systems.'. *J. Microsc.* **158**: 177185.

[24] Conan V., Howard C.V., Jeulin D., Renard D. & Cummins P.(1990).
'Improvements of 3D confocal microscope images by geostatistical filters.'. *Trans Roy Mic Soc*. **1**: 281284.

[25] Gesbert S., Howard C.V., Jeulin D. & Meyer F.(1990).
'The use of basic morphological operations for 3D biological image analysis.'. *Trans Roy Mic Soc* **1**: 293296.

[26] Zhao H.Q., Browne M.A. & Howard C.V. (1990).
'Digital stereology - a comparative study of digital stereological surface density estimators.'. *Trans Roy Mic Soc* **1**: 315319.

[27] Behnam Rassoli M., Herbert L.C., Howard C.V., Pharoah P.O.D. & Stanisstreet M. (1990).
'Effect of propyl thiouracil (PTU) treatment during prenatal and early postnatal development on the neocortex of rat pups.'. *Neuroendocrinology* **53**(4): 321327.

[28] Hinchliffe S.A., Chan Y.F., Howard C.V., & van Velzen D. (1990).
'An assessment of volume-weighted mean nuclear volume estimates as a prognostic index for neuroblastoma.' *Pediatric Pathology* **10**: 973986.

[29] Hinchliffe S.A., Sargent P.H., Howard C.V., Chan Y.F. & van Velzen, D. (1991).
'Human Intrauterine Renal Growth expressed in absolute number of glomeruli assessed by the 'Disector' method and Cavalieri principle.'. *Lab Invest* **64**: 777784.

[30] Cruz-Orive L.M., & Howard C.V. (1991).
'Estimating the length of a bounded curve in 3D using total vertical projections.' *J. Microsc*. **163**: 101114.

[31] Zhao H.Q., Browne M.A. & Howard C.V.(1991).
'Digital probes for three dimensional microstructural analysis.'. *Machine Vision and Application* **4**: 255261.

[32] Roberts N., Howard C.V., Cruz-Orive L.M. & Edwards R.H.T. (1991).
'The application of the total vertical projections for the unbiased estimation of the length of blood vessels and other structures by magnetic resonance imaging.' *Magnetic Resonance Imaging* **9**: 917-925.

[33] Howard C.V., & Sandau K (1992).

'Measuring the surface area of a cell by the method of spatial grid with a CSLM - A demonstration.' *J. Microsc*. **165**: 183-188

[34] Hinchliffe S.A., Sargent P.H., Howard C.V., Chan Y.F., Hilton J.L., Rushton D.I. and van Velzen D. (1992).
'Medullary Ray Glomerular Counting as a method of assessment of human nephrogenesis.'. *Pathology Research and Practice* **188**: 775782.

[35] Hinchliffe S.A., Lynch M.R.J., Sargent P.H., Howard C.V., van Velzen D. (1992).
'The effect of human intrauterine growth retardation on the development of renal nephrons.' *British Journal of Obstetrics and Gynaecology* **99**: 296301.

[36] Howard C.V., CruzOrive L.M. & Yaegashi H. (1992).
'Estimating neuron dendritic length in 3-D from total vertical projections and from vertical slices.'. *Acta Neurologica Scandinavica* **137**: 1419.

[37] Conan V., Gesbert S., Howard C.V., Jeulin D., Meyer F. & Renard D. (1992).
'Geostatistical and morphological methods applied to three-dimensional microscopy.' . *J. Microsc*. **166**: 169185.

[38] Cummins D., Moss M.C., Jones C.L., Howard C.V., & Cummins P.G. (1992).
'Confocal microscopy of dental plaque development.'. *Binary Comput Microbiol* **4**(3): 8692.

[39] Zhao H.G., Browne M.A. & Howard C.V. (1992).
'Automatic 3-D disector sampling for volume distribution measurements.' *Acta Stereol* **11**: 215220.

[40] Jiang Y., Howard C.V., Prasad B., Dangerfield P. & van Velzen D. (1992).
'Segmentation of human hand X-ray images for bone age analysis.'. *Acta Stereol* **11**: 747752.

[41] Moss M.C., Veiro J.A., Singleton S., Gregory D.P., Birmingham J.J., Jones C.L., Cummins D., Miller R.M., Sheppard R.C., Howard C.V., & Bhaskar N. (1993).
'Applications of confocal laser scanning microscopy in in-situ mapping.'. *The Analyst* **118**: 19.

[42] Howard C.V., Jolleys G., Stacey D., Fowler A., Browne M.A. & Wallen P. (1993).
'Measurement of total neuronal volume, surface area and dendritic length following intracellular physiological recording.'. *Neuroprotocols* **2**: 113120.

[43] Hinchliffe S.A., Howard C.V., Lynch M.R.J., Sargent P.H., Judd B.A. & van Velzen D.(1993)

'Renal developmental arrest in sudden infant death syndrome.'. *Paediatric Pathology* **13**: 333343.

[44] Baddeley A.J., Moyeed R.A., Howard C.V. & Boyde A. (1993).
'Analysis of a three dimensional point pattern with replication.'. *Appl. Statist*. **42**: 641668.

[45] Yaegashi H., Howard C.V., McKerr G. & Burns A. (1993).
'Stereological estimation of the total number of neurons in the asexually dividing tetrahyridium of Mesocestoides corti.' *Parasitology*, **106**: 177183.

[46] Ibiwoye M.O., Howard C.V., Sibbons P.D., Hasan M. & van Velzen D. (1993).
'Cerebral malaria in the Rhesus monkey: Observations on the host pathology.' *Journal of Comparative Pathology*. **108**: 303310.

[47] Ibiwoye M.O., Sibbons P.D., Howard C.V., Hasan M., Mahdi A.A., Desalu A.B.O. & van Velzen D. (1993).
'Cerebral malaria in the Rhesus monkey (Macaca mulatta): Light and electron microscopic changes in blood cells and cerebrovascular endothelia.' *Comparative Haematology International* **3**: 153158.

[48] Boon M.E., Howard C.V., & van Velzen D.(1993).
'PCNA independence of KI67 expression in HPV infection.' *Cell Biology International*.**17**: 10011004

[49] Markova E., Markov D., van Velzen D. & Howard C.V. (1993).
'Grey level image analysis using a topological tree of connected figures of serial binary sections.' *Acta Stereol*. **12**: 227232.

[50] Allen S.J., Pierro A., Cope L., Macleod A., Howard C.V. & van Velzen D. (1993).
'Glutamine supplementated parenteral nutrition in a child with short bowel syndrome.' *J. Pediatric GastroEnterology and Nutrition*. **17**:329332.

[51] Ball L.M., Hinchliffe S.A., Howard C.V., Jiang Y. & van Velzen D. (1993).
'Automated image analysis of silver stainable nucleolar organising regions in childhood acute lymphoblastic leukaemia.'. *Cancer Therapy and Control* **4** Suppl.4: 331337.

[52] Khine M.M., Aung W., Sibbons P.D., Howard C.V., Clapham E., McGill F. & van Velzen D. (1994)
'Analysis of relative proliferation rates of Wilms tumour components using PCNA and MIB-equivalent antigen immunostaining and assessment of mitotic index.' *Laboratory Investigation* **70**: 125129.

[53] Ball L.M., Pope J., Eccles P., Howard C.V., & van Velzen D. (1994).

'PCNA/Ki-7 dissociation in childhood lyphoblastic leukaemia.' *Cell Biology International*, **18**: 869874.

[54] Ball L.M., Hinchliffe S.A., Bataringaya J., Howard C.V., England K.E. & van Velzen D. (1994).
'Relationship between MDR- gene expression and Ag-NOR pattern in childhood lymphoblastic leukaemia.'. *Cancer Therapy and Control*, **4**: 39.

[55] Ibiwoye M.O., Sibbons P.D, Howard C.V., & van Velzen D. (1994).
'Immunocytochemical study of vascular barrier antigen in the developing rat brain.' *J Comp Path*. **111**: 4353.

[56] Browne M.A., Howard C.V., Jolleys G., Stacey D. (1994).
'A generalized terminology for multidimensional microscopy.' *J. Microscopy*, **175**: 90

[57] Cox H., Walker C. & Howard C.V. (1995).
'Thick bone section preparation using a silicon-rubber-based sealant.'. *J. Microscopy* **177**: 9092.

[58] Howard C.V., van Velzen D., Ansari T., Li Y. & Pahal N. (1995).
'3-D Unbiased stereological measurements using conventional light microscopy, applied to the study of human intra-uterine growth retardation.'. *Zoological Studies* **34**: 109110

[59] Cruz-Orive L.M. & Howard C.V. (1995).
'Estimation of individual feature surface area with the vertical spatial grid.' *J. Microscopy* **178**: 146151.

[60] Hooper P., Smythe E., Richards R.C., Howard C.V., Lynch R.V. & LewisJones D.I. (1995).
'Total number of immunocompetent cells in the normal rat epididymis and after vasectomy.' *Journal of Reproduction and Fertility* **104**: 193198.

[61] Ansari T., Howard C.V., Yan L., Macmillan A., Pahal N., Hinchliffe S.A., Beech D.J., Beddoes L., Friedman K., Sibbons P.D., & van Velzen D. (1995).
'A stereological assessment of IUGR in relation to the brain, the phrenic nerve, and kidneys and the lungs.'. *European Journal of Morphology*. **33** (4): 294-298

[62] Stringer R.L., Ryan J.J., & Howard C.V. (1995).
'Dioxin contamination of workers' blood and wastes at a chlorophenol manufacturing site in the UK.'. *Organohalogen Compounds* **26**: 97-100.

[63] Howard C.V., Fraser W., Stringer R.L., Ryan J.J., van Velzen D., Ansari T., Byrd A.,

Diver M. & Johnston P.A. (1995).
'Reduction of serum testosterone concentration and perterbation of serum immunoglobulin M and G3 concentrations in a group of dioxin exposed workers.' *Organohalogen Compounds* **25**: 8586.

[64] van Velzen D., Krishnan K.R., Parsons K.F., Soni B.M., Fraser M.H., Howard C.V., Vaidyanathan S. (1995).
'Comparative pathology of dome and trigone of urinary-bladder mucosa in paraplegics and tetraplegics.' *Paraplegia,* .**33**: 565-572

[65] Ibiwoye M.O., Sibbons P.D., Hasan M., Howard C.V., Desalu A.B.O., Singhal K.C., van Velzen D. (1995).
'Lipofuscin pigment in cerebellar purkinje neurons and choroid- plexus epithelial-cells of macaque monkeys with plasmodium-knowlesi cerebral malaria - an electron-microscopic observation.' *Journal Of Veterinary Medicine Series B*, **42**:140-146

[66] van Velzen D., Krishnan K.R., Parsons K.F., Soni B.M., Howard C.V., Fraser M.H., Vaidyanathan S. (1995).
'Vesical urothelium proliferation in spinal-cord injured persons - an immunohisto-chemical study of PCNA and MIB.1 labeling.' *Paraplegia*, **33**: 523-529

[67] Crangle K.D., McKerr G., Allen J.M., Howard C.V., Johansson O. (1995).
'The central nervous system of *grillotia erinaceus* (cestoda, trypanorhyncha) as revealed by immunocytochemistry and neural tracing.' *Parasitology Research*, **81**: 152-157

[68] Hinchliffe S.A., Howard C.V., van Velzen D. (1995).
'Maternal smoking and blood-pressure in 7.5 to 8 year old offspring.' *Archives of Disease in Childhood*, **73**: 378.

[69] Burns A.J., Howard C.V., Allen J.M., van Velzen D., McKerr G (1995).
'Stereological estimation of gap junction surface area per neuron in the developing nervous system of the invertebrate Mesocestoides corti.' *Parasitology*, **111**: 505-513.

[70] van Velzen D., Ball L.M., Dezfulian R.A., Southgate A., Howard C.V. (1996).
'Comparative and experimental pathology of fibrosing colonopathy.' *Postgraduate Medical Journal*, **72**: 39-48.

[71] Vaidyanathan S., van Velzen D., Krishnan K.R., Parsons K.F., Soni B.M., Woolfenden A., Howard C.V. (1996).
'Nerve fibres in urothelium and submucosa of neuropathic urinary bladder: an immunohistochemical study with S-100 and neurofilament.' *Paraplegia*, 34:137-151.

[72] Bains R.K., Sibbons P.D., Murray R.D., Howard C.V. van Velzen D. (1996).
'Stereological estimation of the absolute number of glomeruli in the kidneys of the lamb.' *Research in Veterinary Science,* **60**:122-125.

[73] Hinchliffe S.A., Smith M.D., Boon M.E., Howard C.V., van Velzen D. (1996).
'Evidence for dissociation of histone messenger-RNA expression from cellular proliferation in cutaneous human papilloma virus infection.' *Journal of Pathology*, **178**: 249-254.

[74] van Loosen J., Yiang J., Howard C.V., van Zanten G.A., Verwoerd-Verhoef H.L., Verwoerd C.D.A., van Velzen D. (1996).
'Nasal cartilage maturation assessed by automated computer assisted image analysis.' *Adv ORL*, **51**: 51-60

[75] Sibbons P.D., Aylward G.L., Howard C.V., van Velzen D. (1996)
'A quantitative immunocytochemical analysis of total surface area of blood-brain barrier in developing rat brain.' *Comparative Haematology International,*, **6** 214-220.

[76] van Loosen J., van Zanten G.A., Howard C.V., Verwoerd-Verhoef H.L., van Velzen D., Verwoerd C.D.A. (1996). 'Growth characteristics of the human nasal septum.' *Rhinology*, **34**: 78-82.

[77] Reed M.G., and Howard C.V. (1997).
'Edge corrected estimators of the nearest-neighbour distance distribution function for three-dimensional point patterns.' *J. Microscopy*, **186**: 177-184.

[78] Howard C.V. (1997).
'Quantitative microscopy in studies of intrauterine growth retardation.' Editorial, *Journal of Pathology*, **183**: 129-130

[79] Reed M.G., Howard C.V., Shelton C.G. (1997).
'Confocal imaging and second-order stereological analysis of a liquid foam.' *J. Microscopy*, **185**: 313-320

[80] Casson I.F., Clark C.A., Howard C.V., McKendrick O., Pennycook S., Pharoah P.O.D., Platt M.J., Stanisstreet M., van Velzen D., Walkinshaw S. (1997).
'Outcomes of Pregnancy in insulin dependent diabetic women: result of a five year population cohort study' *British Medical J* **315**: 275-8

[81] Ho M.W., Traavik T., Olsvik R., Midtvedt T., Tappeser B., Howard C.V., von Weizsacker C. & McGavin G. (1998).
'Gene Technology and Gene Ecology of Infectious Diseases.' *Microbial Ecology in Health and Disease* **10**: 33-59

[82] Ricketts S.A., Sibbons P.D., Howard C.V., van Velzen D. (1998).
'Bacterial translocation in pre-necrotising enterocolitis intestinal mucosa assessed by confocal laser scanning microscopy.' *Journal of Cellular Pathology,* **3**: 17-26.

[83] Reed M.G., Howard C.V. (1998).
'Surface-weighted star volume: concept and estimation.' *Journal of Microscopy,* **190**: 350-356.

[84] McLean W.G., Holme A.D., Janneh O., Southgate A., Howard C.V. and Reed M.G. (1998) 'The effect of benomyl on neurite outgrowth in mouse NB2A and human SH-SY5Y neuroblastoma cells in vitro.' *Neuro-Toxicology* **19**: 629-632

[85] Norton T.A., Thompson R.C., Pope J., Veltkamp C.J., Banks B., Howard C.V., Hawkins S.J. (1998).'Using confocal laser scanning microscopy, scanning electron microscopy and phase contrast light microscopy to examine marine biofilms.' *Aquatic Microbial Ecology*, , **16**: 199-204

[86] Howard C.V., D.J., Reed M.G., Moss M.C. (1999).
'A double microscope for the efficient application of the physical disector - the tandem projection microscope.' *Journal of Cellular Pathology*, **4**: 79-85.

[87] Brotchie D., Birch M., Roberts N., Howard C.V., Smith V.A., Grierson I. J.(1999).
'Localisation of connective tissue and inhibition of autofluorescence in the human optic nerve and nerve head using a modified picrosirius red technique and confocal microscopy.' *Neuroscience Methods*, **87**: 77-85

[88] Reed M.G., Howard C.V. (1999).
'Stereological estimation of covariance using linear dipole probes.' *J. Microscopy*. **195**: 96-103

[89] Brotchie D, Roberts N., Birch M., Hogg P., Howard C.V. and Grierson I. (1999).
'Characterisation of Ocular Cellular and Extracellular Structures Using Confocal Microscopy and Computerised Three-Dimensional Reconstruction.' Confocal Microscopy,, *Methods in Enzymology*, **307**: 496-513.

[90] Howard C.V. and Saunders P.T. (1999).
'Sensible precautions make good science.' Letter: *Nature* **401**: 207.

[91] Beech D.J., Howard C.V., Reed M.G., Sibbons P.D., van Velzen D. (2000).
'Unbiased and efficient estimation of the total number of terminal bronchiolar duct endings in lung: A modified physical disector.' *J. Microscopy*. **197**: 36-45.

[92] Beech D.J., Sibbons P.D., Howard C.V., van Velzen D. (2000).
'Terminal bronchiolar duct ending number does not increase post-natally in normal infants.' *Early Human Development*, **59**:193-200.

[93] Beech D.J., Sibbons P.D., Howard C.V., van Velzen D. (2000).
'Renal developmental delay, expressed by reduced glomerular number, and its association with growth retardation in victims of sudden infant death syndrome and "normal" infants.' *Pediatric and Developmental Pathology*, **3**: 450-454.

[94] Ansari T, Sibbons PD, Howard CV (2001). Estimation of Mean Nuclear Volume of Neocortical Neurons in Sudden Infant Death Syndrome Cases Using the Nucleator Estimator Technique Biol Neonate **80**:48-52 (DOI: 10.1159/000047119)

[95] Reed MG, Shanks E, Beech DJ, Barlow L, Howard CV. (2001).
'Stereological estimation of eye volume using the Pappus method.' *Journal of Microscopy*, **3**: 473-479.

[96] Ansari T, Sibbons PD, Howard CV (2001).
Estimation of mean nuclear volume of neocortical neurons in sudden infant death syndrome cases using the nucleator estimator technique. *Biol Neonate* **80**: 48-52.

[97] Platt MJ, Stannisstreet M, Howard CV, Casson IF, Walkinshaw S, Pennycook S, McKendrick O. (2002).
'St Vincent's declaration 10 years on: outcome of diabetic pregnancies.' *Diabetic Med* **19**: 216-220.

[98] Axelrad JC, Howard CV, McLean WG. (2002).
'Interactions between pesticides and components of pesticide formulations in an in vitro neurotoxicity test.' *Toxicology*, **173**, 259-268.

[99] Hill AJ, Howard CV, Cossins AR (2002).
Efficient embedding technique for preparing small specimens for stereological volume estimation: zebrafish larvae. *J Microsc*. **206**: 179-181.

[100] Kelsall A, Fitzgerald AJ, Howard CV, Evans RC, Singh R, Rhodes JM, Goodlad RA (2002).
Dietary lectins can stimulate pancreatic growth in the rat. *International Journal of Experimental Pathology* **83**: 203-208.

[101] Dhaliwal GS, Murray RD, Rees EM, Howard CV, Beech DJ (2002).

Quantitative unbiased estimates of endometrial gland surface area and volume in cycling cows and heifers. *Research In Veterinary Science* **73**: 259-265.

[102] Axelrad JC, Howard CV, McLean WG. (2003).
'The effects of acute pesticide exposure on neuroblastoma cells chronically exposed to diazinon' *Toxicology* **185**: 67-78.

[103] Hill A, Howard CV, Strahle U, Cossins A (2003).
'Neurodevelopmental defects in zebrafish (Danio rerio) at environmentally relevant dioxin (TCDD) concentrations.' *Toxicological Sciences* **76**: 392-399.

[104] Howard CV, de Yanes GS (2003)
'Telomere length and mortality' Lancet 361: 1224

[105] Pennycook FR, Diamand EM, Watterson A, Howard CV.(2004).
Modeling the Dietary Pesticide Exposures of Young Children. *International Journal of Occupational and Environmental Health* **10**: 304-309.

[106] Stott A, Nik H, Platt MJ, Casson IF, Walkisnshaw, Howard V, Stanisstreet M, Pennycook S.(2004).
Glycaemic control in pregnant women with type 1 diabetes and fetal macrosomia. *Practical Diabetes International* **21**: 209-240.

[107] Hill, A., Howard, V., and Cossins, A. (2004). Characterization of TCDD-induced craniofacial malformations and retardation of zebrafish growth. J Fish Biol 64, 911-22.

[108] Cranston A, Howard L, Howard CV (2004).
Quantitative phenotyping as an efficient means to estimate C-cell number in a knock-in mouse model of MEN2B. *Transgenic Research*, **13**: 339-348.

[109] Newby J & Howard C.V. (2005). Environmental influences in cancer aetiology.*J. Nutr. And Envir. Med*. **15**: 56-114.

[110] Lau K, McLean WG, Williams DP, and Howard CV. (2006).
Synergistic interactions between commonly used food additives in a developmental neurotoxicity test. *Toxicological Sciences*.**90**: 178-187.

[111] Sneddon JC, Boomker E and Howard CV (2006). Mucosall surface area and fermentation activity in the hind gut of hydrated and chronically dehydrated working donkeys. *J. Anim. Sci*. **84**: 119-124.

[112] Ellis KA, Innocent G, Grove-White D, Cripps P, McLean WG, Howard CV, and Mihm M (2006). Comparing the fatty acid composition of organic and conventional milk. *Journal of Dairy Science*. **89**: 1938-1950.

[113] E.M. Kannekens, R.D. Murray, C.V. Howard and J. Currie. (2006). Stereological method for estimating feto-maternal exchange surface area in the bovine placentome at 135 days gestation. *Res. Vet. Sci*.**81**: 120-127.

[114] Belpomme D, Ingaray P, Sasco AJ, Newby JA, Howard CV, Clapp R and Hardell L. (2007) The growing incidence of cancer: Role of lifestyle and screening detection (Review). *Int. J. Oncol*. **30**: 1037-1049.

[115] JG Koppe, A Bartonova, G Bolte, M-L Bistrup, C Busby, M Butter, P Dorfman, A Fucic, D Gee, P van den Hazel, CV Howard, M Kohlhuber, M Leijs, C Lundqvist, H Moshammer, R Naginiene, R Ronchetti, G Salines, G Schoeters, GT Tusscher, MK Wallis, M Zuurbier & P Nicolopoulou-Stamati. (2006). Exposure to multiple environmental agents and their effect. *Acta Paediatrica*. **Suppl 453**: 106-113.

[116] Ellis K.A., Innocent G. T., Mihm M., Cripps P., McLEan W.G., Howard C.V. and Grove-White D. (2007). Dairy cow cleanliness and milk quality on organic and conventional farms in the UK. *J. Dairy Science*. **89**: 1938-1950

[117] Ellis KA, Monteiro A, Innocent GT, Grove-White D, Cripps P, McLean WG, Howard CV, Mihm M (2007). Investigation of the vitamins A and E and beta-carotene content in milk from UK organic and conventional dairy farms. *J. Dairy Res* **74**: 484-491.

[118] Newby JA, Busby CC, Howard CV and Platt MJ (2007). The cancer incidence temporality index: An index to show the temporal changes in the age of onset of overall and specific cancer (England and Wales, 1971-1999). *Biomedicine and Pharmacotherapy*. **61**: 623-630

[119] Sneddon JC, Ritruechait P, Staats de Yanes G and Howard CV (2008) Seasonal influences on quantitative changes in sweat-associated anatomy in native and thoroughbred horses. *Veterinary Dermatology* **19**: 163-173

[120] Irigaray P, Newby JA, Clapp R , Hardell L , Howard CV, Montagnier L, Epstein S , Belpomme D. (2007) Lifestyle-related factors and environmental agents causing cancer: An overview. *Biomedicine & Pharmacotherapy* **61**: 640-658

[121] Barnes CA, O'Hagan BMG, Howard CV& McKerr G (2007). Verification of cell viability at progressively higher scanning forces using a hybrid atomic force and fluorescence microscope. *Journal of Microscopy*, **22**: 185–189

[122] Myllynen PK, Loughran ML, Howard CV, Sormunend R, Walshe AA, Vähäkangas KH (2008) Kinetics of gold nanoparticles in the human placenta. *Developmental Toxicology* **26**: 130-137

[123] Barnes A, Elsaesser A, Arkusz J, Smok A, Palus J, Leshniak A, Salvati A, Hanrahan JP, de Jong WH, Dziubatowska E, Stepnik M, Rydzynski K, McKerr G, Lynch I, Dawson KA, and Howard CV (2008) Reproducible Comet Assay of Amorphous Silica Nanoparticles Detects No Genotoxicity *Nano Lett*., **8** (9), 3069-3074

[124] Darragh G. McArt; Gillian R. Wasson; George McKerr; Kurt Saetzler; Matt Reed; C. Vyvyan Howard (2009) Systematic random sampling of the comet assay. *Mutagenesis*; **24**: 373-378.

[125] Van Hoecke K, Quik JK, Mankiewicz-Boczek J, Deschamphelaere KC, Elsaesser A, Vandermeeren P, Barnes C, McKerr G, Howard CV, Vandemeent D, Rydzynski K, Dawson KA, Salvati A, Lesniak A, Lynch I, Silbersmit G, J'Orndesamber B, Vincenze L, and Janssen CR (2009). Fate and Effects of CeO2 Nanoparticles in Aquatic Ecotoxicity Tests. *Environ. Sci. Technol.* **43**: 4537–4546

[126] Park MVDZ, Annema W, Salvati A, Lesniak A, Elsaesser A, Barnes C, McKerr G, Howard CV, Lynch I, Dawson KA, Piersma AH, de Jong WH (2009). Toxicity of silica nanoparticles of different sizes in the mouse embryonic stem cell test. *Toxicology and Applied Pharmacology* **240**: 108–116

[127] Reed MG, Howard CV & Staats de Yanes G (2010). One-Stop Stereology: the estimation of 3D parameters using Isotropic Rulers. *Journal of Microscopy*, **239**: 54-65.

[128] Elsaesser A, Taylor A, Staats de Yanes G, McKerr G, Kim E-M, O'Hare E & Howard CV (2010). Quantification of nanoparticle uptake by cells using microscopy and analytical techniques. *Nanomedicine*, **5**: 1477-1457.

[129] Hazekamp J, Doherty S, Elsaesser A, Barnes CA, O'Hagan B, McKerr G & Howard CV (2011). Focussed ion beam milling at grazing incidence angles. *Journal of Microscopy*, **242**: 104-110.

[130] Park MV, Verharen HW, Zwart E, Hernandez LG, van Benthem J, Elsaesser A, Barnes C, McKerr G, Howard CV, Salvati A, Lynch I, Dawson KA & de Jong WH. (2011) Genotoxicity evaluation of amorphous silica nanoparticles of different sizes using the micronucleus and the plasmid LacZ gene mutation assay. *Nanotoxicology*, **5**: 168–181

[131] Elsaesser A, Barnes CA, McKerr G, Salvati A, Lynch I, Dawson KA & Howard CV (2011). Quantification of nanoparticle uptake by cells using an unbiased sampling method and electron microscopy. *Nanomedicine.* 6: 1189-1198

[132] Park M, Lynch I, Ramírez-García S, Dawson K, de la Fonteyne L, Gremmer E, Slob W, Briedé J, Elsaesser A, Howard CV, Loveren H, de Jong W. (2011). In vitro evaluation of cytotoxic and inflammatory properties of silica nanoparticles of different sizes in murine RAW 264.7 macrophages. *J. Nanoparticle Research* **13,** 6775-6787

[133] Elsaesser A, Howard CV (2012), Toxicology of nanoparticles , *Adv. Drug Deliv. Rev.* **64**: 129–137

[134] Antoniou M, Habib MEM, Howard CV, RC Jennings RC, Leifert C, Nodari RO, Robinson CJ and J Fagan J (2012). Teratogenic Effects of Glyphosate-Based Herbicides: Divergence of Regulatory Decisions from Scientific Evidence *.J Environ Anal Toxicol*, **S:4** http://dx.doi.org/10.4172/2161-0525.S4-006

[135] Van Hoecke K, De Schamphelaere KAC, Ali Z, Zhang F, Elsaesser A, Rivera Gil P, Parak WJ, Smagghe G, Howard CV and Janssen CR (2013). Ecotoxicity and uptake of polymer coated gold nanoparticles. *Nanotoxicology* **7(1)**: 37-47

[136] Hansen SF, Howard CV, Martuzzi M and Depledge M (Eds). *Nanotechnology and human health: Scientific evidence and risk governance.* Report of the WHO expert meeting 10–11 December 2012, Bonn, Germany. Copenhagen, WHO Regional Office for Europe, 2013.

[137] Michaelis S, Burdon J, Howard CV (2017) Aerotoxic syndrome: A new occupational disease? *Public Health Panorama* (WHO), **3**, 141-356.

[138] Busby C, Howard CV (2017). Re-Thinking Biology—I. Maxwell's Demon and the Spontaneous Origin of Life. *Advances in Biological Chemistry*, **7**, 170-181.

[139] Howard CV, Michaelis S, Watterson A (2017). The Aetiology of 'Aerotoxic Syndrome'- A Toxico- Pathological Viewpoint. *Open Access Journal of Toxicology*, **1, 5**

[140] Howard CV, Johnson DW, Morton J, Michaelis S, Supplee D, et al. (2018) Is a Cumulative Exposure to a Background Aerosol of Nanoparticles Part of the Causal Mechanism of Aerotoxic Syndrome? J Nanomed Nanosci: JNAN-139. DOI: 10.29011/ JNAN-139. 100039

BOOKS

Howard C V and Reed M G
Unbiased Stereology. 1st Edition (Bios Scientific Publishers, Oxford UK, 1998, ISBN 1-85996-071-5) 246

Maynard R L & Howard C V (Author/Editors)
Particulate Matter: Properties and Effects upon Health. (Bios Scientific Publishers, Oxford UK, 1999, ISBN 1-85996-172-X) 184

Nicolopolou-Stamati P, Hens L and Howard C V (Author/Editors)
Health Impacts of Waste Management Policies. (Kluwer Academic Publishers, Dordrecht, The Netherlands, 2000, ISBN 0-7923-6362-0) 326

Nicolopolou-Stamati P, Hens L and Howard C V (Author/Editors)
Environmental Health Aspects of Endocrine Disruptors. (Kluwer Academic Publishers, Dordrecht, The Netherlands, 2001, ISBN 0-7923-7056-2) 376

Nicolopolou-Stamati P, Hens L, Howard C V and Van Larebeke N (Author/Editors)
Cancer as an Environmental Disease. (Kluwer Academic Publishers, Dordrecht, The Netherlands, 2004, ISBN 1-4020-2019-8) 236

Howard C V and Reed M G
Unbiased Stereology. 2nd Edition (Garland Bios Scientific Publishers, Abingdon UK, 2005, ISBN 1-8599-6089-8) 277.

Nicolopolou-Stamati P, Hens L and Howard C V (Author/Editors)
Environmental Health Aspects of Transport and Mobility. (Springer, Dordrecht, The Netherlands, 2005, ISBN 1-4020-4304-X) 315

Nicolopolou-Stamati P, Hens L and Howard C V (Author/Editors)
Congenital Diseases and the Environment. (Springer, Dordrecht, The Netherlands, 2007, ISBN 1-4020-4830-0) 457

Nicolopolou-Stamati P, Hens L and Howard C V (Author/Editors)
Reproductive Health and the Environment. (Springer, Dordrecht, The Netherlands, 2007, ISBN 1-4020-4828-9) 381

CHAPTERS IN BOOKS & ACADEMIC ARTICLES

Howard C.V.
'The confocal microscope as a geometrical probe.' In Confocal Optical Microscopy 2nd Edition. ed. T Wilson (Academic Press, London, 1990, ISBN 0127572708) 285302

Howard C.V.
'Stereological Techniques in Biological Electron Microscopy .' In Biophysical Electron Microscopy eds. P W Hawkes and U Valdre. (Academic Press, London, 1990, ISBN 0123333555) 479508

Howard C.V.
'The confocal microscope as a tool for probing 3D geometry.'. *Proceedings of SPIE,* 1990, **1245**, Biomedical Image Processing, 4660.

Hall A., Browne M.A. & Howard C.V.
'Confocal microscopy - The basics explained.'. *Proc. Roy. Mic Soc*., 1991, **26/2**: 6370.

Browne M.A., Howard C.V., & Jolleys G.D.
'Principles of stereology.' In Image analysis in histology. Eds. R Wootton, D R Springall and J M Polak (Cambridge University press, 1995, ISBN 0512-43482-3) 96120

Howard C.V., & van Velzen D.
'Foetal susceptibility to permanent damage: as illustrated by organochlorine pollutants.' In Environmental poisoning and the Law. ed. W Sigmund (Environmental Law Foundation, 1995, ISBN 0951607316) 25-40

Markova E.G, Howard C.V., van Velzen D.
'Morphological changes in substantia nigra neurons of infants brains in un-explained cot death (SIDS) and IUGR.' In Impact of Antenatal and Postnatal Environmental Factors on Infant Outcome. ed. P Johnson (Proceedings of the 3rd Congress of the European Society for the Study and Prevention of Infant Deaths, Holywell Press, Oxford, UK, 1995, ISBN 0-9527125-0-4) 53-57

Friedman K., Howard C.V., Sibbons P.D., Yaegashi H., Takahashi T., van Velzen D.
'Alterations of terminal duct complex configuration of the human lung in SIDS related to IUGR' In Impact of Antenatal and Postnatal Environmental Factors on Infant Outcome, ed. P Johnson (Proceedings of the 3rd Congress of the European Society for the Study and Prevention of Infant Deaths, Holywell Press, Oxford UK, 1995, ISBN 0-9527125-0-4) 33-35.

Beech D.J., Sibbons P.D., Howard C.V., van Velzen D.
'Lung gas exchange surface area in IUGR determined by stereological techniques.' In Impact of Antenatal and Postnatal Environmental Factors on Infant Outcome, ed. P Johnson (Proceedings of the 3rd Congress of the European Society for the Study and Prevention of Infant Deaths, Holywell Press, Oxford, UK, 1995, ISBN 0-9527125-0-4) 31-32.

Johnston P., Stringer R., Santillo D., and Howard C.V.
'Hazard, Exposure and Ecological Risk assessment.' In Environmental Management in Practice, eds. B Nath, L Hens, P Compton, and D Devuyst (Routledge, London & New York, 1998, ISBN 0-415-14906-1) 169-187

Antunes, S., Reed, M.G. & Howard, C.V.
'The Optical Fractionator.' In Focus on Multidimensional Microscopy, Vol 1. ed. P.C. Cheng, P.P. Hwang, J.L. Wu, G. Wang and H. Kim (World Scientific, Singapore, 1999, ISBN 981-02-3991-2) 158-168.

Reed M.G. and Howard C.V.
'Unbiased counting rules in Optical Sectioning Light Microscopy.' *Proceedings of the Royal Microscopical Society,* 1999, **34**: 431-438.

Howard C.V., Staats de Yanés G. and Nicolopolou-Stamati P.
'Persistent Organic Chemical Pollution: An Introduction.' In Health Impacts of Waste Management Policies, auth/eds. P Nicolopolou-Stamati, L Hens and C V Howard (Kluwer Academic Publishers, Dordrecht, The Netherlands, 2000, ISBN 0-7923-6362-0) 29-40.

Howard C.V.
'Particulate Aerosols, Incinerators And Health.' In Health Impacts of Waste Management Policies, auth/eds. P Nicolopolou-Stamati, L Hens and C V Howard (Kluwer Academic Publishers, Dordrecht, The Netherlands, 2000, ISBN 0-7923-6362-0) 155-174.

Staats de Yanés G. and Howard C.V.
'Impacts of Inadequate or Negligent Waste Disposal on Wildlife and Domestic Animals: Relevance for Human Health' In Health Impacts of Waste Management Policies, auth/eds. P Nicolopolou-Stamati, L Hens and C V Howard (Kluwer Academic Publishers, Dordrecht, The Netherlands, 2000, ISBN 0-7923-6362-0) 251-281.

Hens L., Nicolopolou-Stamati P., Howard C.V., Lafere J. and Staats de Yanés G.
'Towards A Precautionary Approach for Waste Management Supported by Education and Information Technology.' In Health Impacts of Waste Management Policies, auth/eds. P Nicolopolou-Stamati, L Hens and C V Howard (Kluwer Academic Publishers, Dordrecht, The Netherlands, 2000, ISBN 0-7923-6362-0) 283-310.

Nicolopoulou-Stamati P., Hens L., Howard C.V. and Pitsos M.A.
'Introductory chapter: awareness of the health impacts of endocrine disrupting substances.' In Environmental Health Aspects Of Endocrine Disruptors, auth/eds P Nicolopolou-Stamati, L Hens and C V Howard (Kluwer Academic Publishers, Dordrecht, The Netherlands, 2001, ISBN 0-7923-7056-2) 1-16.

Howard C.V. and Staats de Yanes G.
'Endocrine Disrupting Chemicals: A Conceptual Framework' In Environmental Health Aspects Of Endocrine Disruptors, auth/eds P Nicolopolou-Stamati, L Hens and C V Howard (Kluwer Academic Publishers, Dordrecht, The Netherlands, 2001, ISBN 0-7923-7056-2) 219-250.

Nicolopoulou-Stamati P., Pitsos M.A, Hens L. and Howard C.V.
'A Precautionary Approach To Endocrine Disrupters' In Environmental Health Aspects Of Endocrine Disruptors, auth/eds P Nicolopolou-Stamati, L Hens and C V Howard (Kluwer Academic Publishers, Dordrecht, The Netherlands, 2001, ISBN 0-7923-7056-2) 331-356.

Howard C.V. and Newby J.A.
'Could the increase in cancer incidence be related to recent environmental changes?'. In Cancer as an Environmental Disease, auth/eds Nicolopolou-Stamati P, Hens L, Howard C V and Van Larebeke N (Kluwer Academic Publishers, Dordrecht, The Netherlands, 2004, ISBN 1-4020-2019-8) 39-56.

Nicolopoulou-Stamati P., Howard C.V. and Gaudet B.A.J.'Re-evaluation of priorities in addressing the cancer issue: Conclusions, strategies, prospects'. In Cancer as an Environmental Disease, auth/eds Nicolopolou-Stamati P, Hens L, Howard C V and Van Larebeke N (Kluwer Academic Publishers, Dordrecht, The Netherlands, 2004, ISBN 1-4020-2019-8) 171-196.

Stewart A.G., Wilkinson E and Howard C. V.
Health: A necessity for sustainable development. In 'The World Summit on Sustainable Development, The Johannesburg Conference. Eds. Hens L. and Bhaskar N. (Springer, Dordrecht, The Netherlands, 2005, ISBN 1-4020-3652-3) 151-181.

Howard C.V. and Ikah D.S.K.
Nanotechnology and nanoparticle toxicity: A case for precaution. In Nanotechnology Risk, Ethics and Law. (Eds) Hunt G. and Mehta M.D. (Earthscan, London, 2006, ISBN 1-84407-358-0) 154-166.

DR. C. V. HOWARD'S DECLARATION IN SUPPORT OF CLASS CERTIFICATION

1)Background

This is a report is prepared for the benefit of the Court.[1] I have been asked to prepare a report addressing the effects of opioids on the developing fetus. I understand my duties as an Expert witness pursuant to Part 35 of the Civil Procedure Rules; a statement of truth is enclosed following my opinion below.

I am a medically qualified toxico-pathologist specialising in the problems associated with the action of toxic substances on health, particularly during the period of development in the womb. My PhD Thesis addressed mechanisms of the selective stabilisation of neurons in developing mammalian brain. I am currently Emeritus Professor of Bioimaging at the University of Ulster and have authored/co-authored over 130 peer reviewed scientific papers, predominantly in the field of quantitative developmental toxicology. I append my Curriculum Vitae.

I am a Fellow of the Royal College of Pathologists, Fellow of the Collegium Ramazzini, Past President of the Royal Microscopical Society, Member of the British Society of Toxico-Pathologists, Past President of the International Society of Doctors for the Environment. I served for 6 years as a toxicologist on the United Kingdom Government DEFRA Advisory Committee on Pesticides which was the statutory body responsible for recommending licensing of agro-chemicals. I have addressed the House of Lords Select Committee on Science and Technology investigating the use of nanotechnology in food. More recently I have given evidence to the House of Commons Environmental Audit Committee on the toxicology of neonicotinoid pesticides to pollinating insects. This resulted in their report 'Pollinators and Pesticides' (HC 668, 2012-13).

Toxico-pathologists are skilled in assessing the effects of toxic substances on health. This includes consideration of routes of entry for toxic substances into the body, assessing the relevance of dose and timing of administration, target organ susceptibility, mechanism of action of toxins, types of pathology induced and dose response, with respect to single substances and mixtures. Such expertise is of relevance in this action because it concerns exposure of the fetus during intra-uterine life to opioids taken by the mother. Opioids have been shown to be able to increase the rate of apoptosis in developing neurons and this has a number of long-term sequelae. This pathological mechanism is within the scope and expertise of toxico-pathologists.

2)The Opioid Receptor (OR)

The opioid receptor system is ubiquitous throughout all vertebrate (animals with backbones) life. Opioid receptors consist of a family of four related proteins which are part of a large superfamily, the rhodopsin-like G-protein coupled receptors. Of the four related proteins, three types of opioid receptor unequivocally are associated with the control of pain in animal models.

[1] This report was developed in collaboration with Dr. Christopher Busby.

These are the □(MOR), □ (DOR) and □ (KOR) opioid receptor proteins. There is a fourth opioid receptor protein which is termed the nociception or orphanin (ORL) whose function is less well defined than the other three. The receptors have natural internally produced (endogenous) opioid peptide ligands which include beta-endorphin, met-and leu-enkephalin and dynorphin. The role of the endogenous opioid receptors on normal physiological activity is extensive; in addition to the obvious role in decreasing painful (nociceptive) sensations they are involved in reproduction, growth, development, respiration, blood pressure regulation, renal function, temperature control, hormonal regulation, seizures, stress, immune response, pregnancy and aging.

Phylogeny:
The OR developed at least 450 million years ago, at the time of the evolutionary emergence of vertebrates with jaws. Early in the evolution of animals there was a single opioid receptor. The first round of genome duplication, which occurred early in cordate evolution, produced the ancestral DOR/MOR and ORL/KOR genes. A further round of genome duplication led to the four opioid receptors found in all living vertebrates (Stevens, 2011). These four ORs are ubiquitous throughout vertebrate phylogeny and intimately involved in the control of reward responses and pain modulation as well as controlling aspects of ontogeny.

3)Opioid Drugs: pharmacology and commonality

Opioids have been used to alleviate pain for thousands of years and remain the most important class of pain-relieving drugs. Opioids exert their effects by mimicking naturally occurring substances in the body, called *endogenous opioid peptides* including *endorphins*. The different functions of this system include
(1) the best-known sensory role—prominent in inhibiting sensory responses to pain
(2) a modulatory role in gastrointestinal, endocrine and autonomic functions, and
(3) and an emotional role, evidenced in powerful rewarding and addictive properties.

Therefore, opioid activity is not restricted to pain relieving effects but in addition exhibit powerful and wide-ranging regulatory roles throughout the organism (Gutstein and Akil, 2006; Stein (2016); Stevens 2011).

In order to understand the effects of the opioids we begin with the production and distribution of the endogenous opioid peptides, since it is these which are mimicked by the opioid drugs which interact with the natural receptors (biological switches). The endogenous opioids are in three distinct families, *the enkephalins, the endorphins and the dynorphins* (Gutstein and Akil, 2006). These substances are small peptides which share a common amino terminal sequence of Tyr-Gly-Gly-Phe-Met (or Leu). This has been termed the *opioid motif* and is followed by various C-terminal extensions yielding peptides ranging from 5 to 31 residues (Gutstein and Akil, 2006).
The precursor protein for Beta Endorphin, *prepro-opiomelanocortin* (POMC) is relatively limited within the Central Nervous System, occurring mainly in the arcuate nucleus and nucleus of the tractus solitarius. These neurons project widely to limbic and brainstem areas and to the spinal cord (Gutstein and Akil, 2006).

The endogenous opioids exert their functions at protein receptors distributed in the brain, and these are the points where the opioid drug molecules, whether natural or synthetic also bind and have pharmacological activity. Opioid receptors consist of a family of four closely related proteins belonging to a large family of receptors called the G-protein coupled receptors. Receptors are large protein molecules which selectively bind pharmacological agents in order to switch on cellular activity that results in a measurable change in some aspect of the cell and the organism. The receptor can be seen as a molecular specific switch. It is believed that specific affinity between the active molecule and the receptor, which occurs only for molecules which can affect the receptor switch positively (termed *agonists*) and produce the effect, defines a class of compounds which have commonality. That is, they have the common molecular nature of causing the process being measured to occur (to a greater or lesser extent). This greater or lesser extent is a function of their *activity* and is measurable. Pharmacologists measure the effect, and plot this against the logarithm of the drug Dose. If the substance is acting at the same receptor, the result is a straight line, (for technical reasons which will not be addressed here). They are termed agonist (for the specific receptor). Compounds which bind to the same receptors but do not cause effects, rather they block the effects of the agonist, are termed antagonists. These also help define a common receptor. More recently, genetic approaches have also characterised families of opioid receptors and described their evolution in both mammalian and invertebrate evolution. Therefore, it is possible to say that the opioids and synthetic opioids, whatever their molecular structure, exert their influence at the same receptor(s) and may thus be considered as a common group (Creeley et al, 2013; Gutstein and Akil, 2006).

There are now considered to be three main types of receptor, termed classical types, the μ, κ and δ. All three have analgesic properties, the μ type causes euphoria, decreases respiratory function and gastrointestinal tract transit (constipation), increases feeding, increases sedation, increases release of growth hormone and prolactin, inhibits neurotransmitter release (acetylcholine and dopamine) and has various other peripheral effects. These examples show the profoundly powerful effects throughout the organism which are modulated by the opioids. It also shows how exposure to, and withdrawal from these species exhibits such wide-ranging effects. All three opioid receptors can modulate pre- and post- synaptic Ca++ channels, supress Ca++ influx and thereby attenuate the excitability of neurons and the release of pro-nociceptive neuropeptides (Gutstein and Akil, 2006). This behaviour is revisited below.

Identity and molecular structure of the opioids (https://webbook.nist.gov/cgi/cbook/).

The medicinally developed opioids are in two groups, those derived from natural products by chemical treatment or separation and those developed by chemical synthesis in order to have affinity and access to the various opioid receptors discussed. The search by pharmaceutical companies and others for substances which produced the analgesic and other valuable effects without side effects or the induction of dependence has been largely unsuccessful. Investment in research into substances which would act as treatments for addiction to morphine and the more powerful narcotic opioids resulted in the discovery and use of methadone and buprenorphine. However, these themselves also result in addiction and withdrawal effects. They are the agents of choice in some schemes of treatment for NAS. The molecular structures of all these compounds are designed to have affinity for the opioid receptors and therefore may

be considered to be one group for the purposes of arguing their membership of the "NAS producing group" of chemical substances. Most of them are semi-synthetic compounds made by chemical treatment of morphine itself or morphine alkaloids like Thebaine (Gutstein and Akil, 2006; O'Brien, 2006; Oates, 2006).

The principal opioids of concern in the current discussion are given in Table 1.

Table 1. Principal opioids associated with NAS (*examples of trade preparations*) [https://webbook.nist.gov/cgi/cbook/].

Opioid	**Nature**	**Note**
Morphine	Main alkaloid constituent of opium; historic medicinal compound. *Avinza, Morphabond, Roxanol-T, Kadian, Mscontin*	Natural substance, main member of the opium poppy alkaloids which also include Thebaine Papaverine.
Hydrocodone	derived from morphine alkaloids. Semi synthetic. *Zohydro ER, Hysingla, Anexsia, Co-gesic, Ibudone, Norco*	Semisynthetic hydrogenated codeine derivative and opioid agonist with analgesic and antitussive effects. Hydrocodone primarily binds to and activates the mu-opioid receptor in the central nervous system (CNS).
Hydromorphone	Also called Dilaudid. Hydromorphone is the hydrogenated ketone of morphine, semi synthetic. *Dilaudid, Palladone,*	Hydromorphone selectively binds the mu-opioid receptor which is linked through G-proteins. Binding stimulates the exchange of guanosine triphosphate (GTP) for guanosine diphosphate (GDP) on the G-protein complex and interacts with and inhibits adenylate cyclase located at the inner surface of the plasma membrane. This leads to a reduction in intracellular cyclic 3',5'-adenosine monophosphate (cAMP). Further, voltage-gated potassium channels are activated, thereby causing hyperpolarization and reducing neuronal excitability. In addition, the opening of voltage-gated calcium channels is inhibited, thereby leading to an inhibition of calcium entry and a reduction in the release of various neurotransmitters, including GABA, vasopressin, somatostatin, insulin and glucagons.
Meperidine	Synthetic *Demerol*	Meperidine is a synthetic piperidine ester with opioid analgesic activity. Meperidine mimics the actions of endogenous neuropeptides via opioid receptors, thereby producing the characteristic morphine-like effects on the mu-opioid receptor, including analgesia, euphoria, sedation, respiratory depression, miosis, bradycardia and physical dependence.
Fentanyl	Synthetic *Abstral, Actiq, Fentora, Onsolis, Sublimaze, Duralgesic*	Powerful synthetic opioid 100 times more powerful than morphine in pain relief
Codeine	Derived from morphine by methylation of the phenolic -OH.	Naturally occurring phenanthrene alkaloid and opioid agonist with analgesic, antidiarrheal and antitussive activities. Codeine mimics the actions of endogenous

		opioids by binding to the opioid receptors at many sites within the central nervous system (CNS). Stimulation of mu-subtype opioid receptors results in a decrease in the release of nociceptive neurotransmitters such as substance P, GABA, dopamine, acetylcholine and noradrenaline; in addition, the codeine metabolite morphine induces opening of G-protein-coupled inwardly rectifying potassium (GIRK) channels and blocks the opening of N-type voltage-gated calcium channels, resulting in hyperpolarization and reduced neuronal excitability. Stimulation of gut mu-subtype opioid receptors results in a reduction in intestinal motility and delayed intestinal transit times. Antitussive activity is mediated through codeine's action on the cough center in the medulla
Buprenorphine	Synthetic analogue of Thebaine from poppy alkaloids *Butrans*	Buprenorphine is a morphinane alkaloid that is 7,8-dihydromorphine 6-O-methyl ether in which positions 6 and 14 are joined by a -CH2CH2- bridge, one of the hydrogens of the N-methyl group is substituted by cyclopropyl, and a hydrogen at position 7 is substituted by a 2-hydroxy-3,3-dimethylbutan-2-yl group. It has a role as an opioid analgesic, a mu-opioid receptor agonist, a kappa-opioid receptor agonist and a delta-opioid receptor antagonist.
Methadone	Synthetic *Dolophine, Methadose*	Methadone is a synthetic opioid with analgesic activity. Methadone mimics the actions of endogenous peptides at CNS opioid receptors, primarily on the mu-receptor and has actions similar to those of morphine and morphine-like agents. The characteristic morphine-like effects include analgesia, euphoria, sedation, respiratory depression, miosis, bradycardia and physical dependence. However, the detoxification symptoms between morphine-like agents and methadone differ in that the onset of methadone's withdrawal symptoms is slower, the course is more prolonged and the symptoms are less severe.
Oxycodone	Semi synthetic. Derived from opium alkaloid Thebaine. *Oxaydo, Xtampza ER, Oxycontin, Percodan, Percoset,*	Oxycodone is a semi-synthetic, morphine-like opioid alkaloid with analgesic activity. Oxycodone exerts its analgesic activity by binding to the mu-receptors in the central nervous system (CNS), thereby mimicking the effects of endogenous opioids.
Oxymorphone	Semi synthetic. Now taken off market in USA (2017) *Opana, OpanaER*	A semisynthetic opioid with a potent analgesic property. Oxymorphone hydrochloride binds to and activates opiate receptors, specifically mu-receptors, in the central nervous system (CNS).
Heroin	Semi synthetic. Illegal in USA.	Heroin is a morphinane alkaloid that is morphine bearing two acetyl substituents on the O-3 and O-6 positions. As with other opioids, heroin is used as both an analgesic and a recreational drug.

Summary of pharmacology

The opioid compounds all act at the same biological receptors and mimic natural peptides which have powerful and wide-ranging activity in living systems. Thus, they can be considered a class of chemical drugs both in terms of their pharmacological dosage activity relationships and also their overall chemical structure. They produce common effects, bind to common receptors, the opioid receptors and also have similar chemical structures. They all produce addiction and dependence and cause withdrawal symptoms on removal. Their activity as modulators of neurological signalling make them especially hazardous in adults due to rebound effects but also they are now known to have significant effects on foetal development since they alter the cellular signalling environment. This issue will be considered below.

4)Dependence and withdrawal in opioids: pharmacology (O'Brien, 2006)

All perturbations of homeostatic systems that last a significant length of time result in two responses. The first is acquired tolerance, which results in a situation where larger doses of the stressor (in this case the opioid drug) are required to effect the same physiological response. The second results from the homeostatic pressure developed by the organism to retain the system's biological status prior to the disturbances created by the perturbation, in this case the chronic use of an opioid drug and the changes brought about in the various systems affected by the receptors. This physical dependence is often termed "Rebound" and is a common feature of withdrawal of all drugs which have Central Nervous System (CNS) effects. Thus, CNS hyperarousal results from re-adaptation to the absence of the drug of dependence (O'Brien, 2006). Since the effects produced by the opioids are so widespread (due to the systems that are perturbed through the 3 natural opiate receptors (at least these: more have been described) the withdrawal of the opioid alteration pressure leads to profound and painful mental and physical effects across a wide spectrum of conditions. Examples of the effects seen in adults are given in Table 2. In Table 3 are listed withdrawal effects seen in babies manifesting NAS. Naturally the same rebound effects are manifest in the babies as exist in the adults.

Table 2 Withdrawal effects in adults seen in opioid removal after chronic use **(O'Brien, 2006)**

Regular withdrawal	**Protracted withdrawal (persist up to 6 months after removal of drug)**
Craving for opioids	Anxiety
Restlessness, irritability	Insomnia
Increased sensitivity to pain	Drug craving

Nausea	Pupillary dilation
Cramps	Sweating
Muscle aches	Piloerection
Dysphoric mood	Tachycardia
Insomnia	Vomiting
Anxiety	Diarrhia
	Yawning
	Fever
	Cyclic changes in weight
	Pupil size

Table 3 Observed NAS withdrawal effects (Wolff K, Perez Montenajo R, 2014)

Effect	**Number of studies reporting 1972-2007**
Neurological Excitability	
High pitched crying	8
Irritability	8
Increased wakefulness/sleep disturbance	8
Hyperactive deep tendon reflexes	9
Hypertonia	6
Exaggerated Moro Reflex	3
Tremors	9
Seizures	9
Myoclonic jerks/ opisthotonic posturing	5
Hyperacusis	1
Intraventricular haemorrhage	1
EEG abnormalities	1
Gastrointestinal dysfunction	
Poor feeding	8
Uncoordinated and constant sucking	7
Vomiting	10
Diarrhoea	10
Dehydration	4
Regurgitation	1
Poor weight gain/ weight loss	6
Hyperphagic (2^{nd} week)	0
Excessive salivation	1
Central nervous system	
Increased sweating	8
Yawning	9
Nasal stuffiness	5
Sneezing	9
Tachypnea	6
Mottled skin	5
Fever	7
Temperature instability	3
Other	
Increased REM sleep	2

Skin excoriating/ scratching	5
Tachycardia/ hypertension	1

These effects are common to withdrawal in the case of all the opioids since these substances act on the same receptors. Of course, there are differences in activity between the various opioid drugs, some having very powerful effects at very low doses relative to morphine, the parent substance, and there is also a variation in the length of time the compound has its effect owing to variations in length of binding to receptors and other factors. The drug Methadone has a much longer period of action so it was chosen (or indeed developed) to act as a drug of choice for dealing with withdrawal from the more immediate effects of morphine and in particular the street drug Heroin. Buprenorphine also has a similar long lasting effect and is used as an alternative to Methadone for reducing the withdrawal effects listed in Tables 2 and 3. However, since both these drugs affect the same receptors that cause the withdrawal effects, it is arguable that their use may produce the same conditions that they are intended to treat. A list of analgesic activity for the various opioids is given in Table 4.

Table 4 Equianalgesic doses for some opioids.

Compound	**Route**	**Dose mg**
Codeine	PO	200
Hydrocodone	PO	20-30
Hydromorphone	PO	7.5
Hydromorphone	IV	1.5
Morphine	PO	30
Morphine	IV	10
Oxycodone	PO	20
Oxycodone	IV	10
Oxymorphone	PO	10
Oxymorphone	IV	1
Fentanyl	Nasal spray/ lozenge	0.1-0.2

5)Apoptosis

It is important to introduce an aspect of basic biology which is of fundamental significance in fetal development. There are two ways in which cells in multicellular organisms can die. One is called ‘necrosis’ – for example if one of the coronary arteries blocks and deprives the heart muscle of oxygen, it will die by necrosis – which is a pathological process. The other mechanism is called ‘apoptosis’, otherwise known as ‘programmed cell death’. This is part of normal biology, particularly during development. For example, almost all individuals had a tail at one stage of fetal life but in almost everybody it melts away by the process of programmed cell death. The hands were solid discs of tissue in their early development but the fissures between the digits appear because of apoptosis remodelling the original disc. There are three basic functions that apoptosis serves:

1) Phylogenetic apoptosis – deletion of vestigial structures
2) Histogenetic apoptosis – controlling cell numbers in the body

3) Morphogenetic apoptosis – remodelling structures

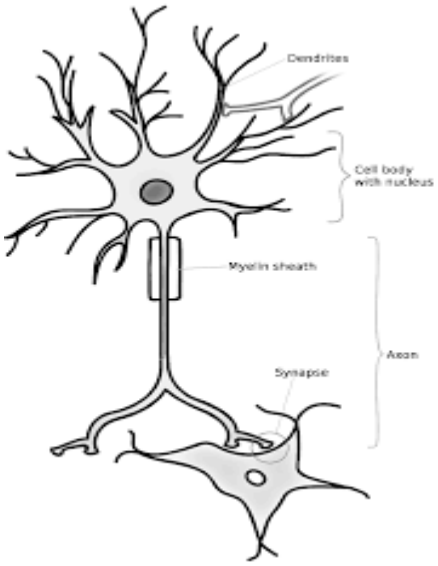
Histogenetic apoptosis is the predominant mode found in the adult. Phylogenetic and morphogenetic apoptosis predominate in the fetus and are indispensable for normal development. Sub optimal brain development and gross malformations (birth defects) have been associated with perturbations of apoptosis.

The Central Nervous System (CNS)

It is important that animal experimental data, as well as human data, is considered when addressing the impact of opioids on developing human brain. Opioid receptors are present in a number of different brain regions and there are several possible mechanisms that opioid exposure could perturb brain development (Yanai et al., 2003). Alterations in the migration and survival of neurons in rat embryos has been demonstrated with exposure to opiates (Harlan, R. E. & Song, D. D., 1994). In vitro studies on human fetal neurons and microglia responses to morphine have been shown to have increased levels of apoptosis [Hu et al., 2002). Reduction in anatomical volumes and cortical thickness when compared to controls in children with heroin and polysubstance exposure

The central nervous system is particularly vulnerable to toxic insult for a number of reasons. The nerve cells that are a component of the adult brain have to last a life time. The adult neuron has a cell body from which a single axon (final common pathway) arises and this conducts a pulse modulated signal on to the next nerve cell in the chain. Whether or not a neuron depolarises to produce the next pulse in the signal chain going down the axon depends on an averaging of all synaptic inputs, excitatory and inhibitory, over the whole receptive surface of the nerve cell, which includes the cell body and the dendritic tree.

Many other organs in the body, for example the liver, can repair by cell proliferation. This does not apply to the nerve cells in the adult CNS, by far the majority of which cannot reproduce themselves. This statement does not hold true for the fetal brain nerve cells, as will be outlined below. The CNS has a very high metabolic rate and neurons have to maintain their microstructures over long distances. For example the axon, which carries outgoing signals from the neuron can be over 1 meter long. To maintain such structures in a healthy state there is a mechanism called ‘axonal transport’ which will deliver a number of substances and structures – in both directions to and from the neuron cell body. Transmitter substances help to deliver information across synapses to the next neurons in the neuronal chain by acting in either an excitatory or an inhibitory manner. The influence of a particular synaptic input onto a neuron will depend on its position on the target neuron and on the firing rate of the axon. ‘Neurotrophins’ are also secreted across the synapse and are essential to maintain the target neurons in good health. Mitochondria are the ‘powerhouses’ in which glucose is metabolised and maintain the high metabolic rate essential for neuronal health, even in the most distant parts of the nerve cell. ‘Neuromodulators’ are a class of biomolecules, to which the endogenous opioids belong, modify the action of transmitter substances at synapses.



6)The relevance of perturbing apoptosis in fetal brain

There are of the order 1015 connections between nerve cells in the adult human central nervous system (CNS). However, humans only have ~ 20,000 genes, which in addition also have to control many other aspects of development. This inevitably means that the development of the brain cannot be determined by the genome alone – there is a massive numerical mismatch. Therefore, nature has evolved a method of arriving at an intact functioning CNS which depends upon a highly probabilistic (chance) based mechanism, which is relatively loosely specified by the genes. The genes control the overall global form of the brain while, at the local level, whether developing cells live or die is decided predominantly by chance.

The importance of synapse formation in the development of brain circuitry was first posited by Changeaux and Danchin (1976) in their theory of 'selective stabilisation'. A more contemporary review has been written by Tau & Peterson (2010). Selective stabilisation involves the loss of a proportion the neurons in a developing brain region, based upon their functional status during critical developmental windows.

In the developing nervous systems of animals (including humans) there is an overproduction of neuroblasts (immature potential neurons) – often more than twice as many as will be finally required in the adult. These start to push out neurites (fibre-like feelers) that make contact with other developing nerve cells in that brain region. Some of the contacts they make at random will be excitatory and others will be inhibitory. At certain developmental times, cell signalling instructions will be broadcast within regions of the CNS and those nerve cells with the correct physiological properties (for example the firing rate of the nerve cell) will carry on with their normal development and the other developing nerve cells which are either under-responsive or over-responsive will undergo apoptosis and melt away. This process is known as 'selective stabilisation'. From this description it can be appreciated that this is largely based on chance connections between too many potential neurons and it leads to a stable functioning circuitry within the CNS with minimal deterministic instructions at the local level from the genes. The mechanism(s) controlling apoptosis in the developing fetal

brain are incompletely understood. However, many cell signalling molecules –including transmitter substances, neuromodulators and hormones are known to be involved. An important harbinger of impending apoptosis in a cell is the appearance of Fas protein being expressed on the cell surface. When this binds with Fas protein ligand (FasL) apoptosis is initiated.

From all of the above it is possible to appreciate how a drug that stimulates nerve cells could act as an 'excitotoxin' in the fetal brain while a neuronal depressant would work the other way. Both of these scenarios result in increases in the proportion of nerve cells undergoing cell death though apoptosis during windows of vulnerability as fetal development progresses. The final outcome in the adult, if this happens, will be sub-optimal development. Reed et al (2010) provide a morphological example. Opioids appear to increase the rate of apoptosis in fetal brain. This is a potential mechanism for neurological damage in the fetus.

Important points to note are:

1) Adverse changes incurred through increased apoptosis are irreversible
2) They generally take place at toxicant concentrations orders of magnitude lower than required to produce damage in an adult.
3) The timing of the toxic insult in the fetal developmental timetable is critical, as it passed through sequential windows of vulnerability

The effects of prenatal exposure to opioids has been reviewed by Anand and Cambell-Yeo (2015). It leads to changes in the temporal sequencing and quality of myelination by disrupting oligodendrocyte development (Svensson et al, 2008). It also decreases the dendritic growth (Nassogne et al, 1885)) and branching pattern complexity (Broussard et al, 2011) of pyramidal neurons in the cortex and suppresses cell proliferation and neuronal migration to the cortical plate. These effects may reduce regional brain volumes in the basal ganglia (McCarthy, 2015) and other brain areas (Yazdy et al, 2015), with long-term changes in subsequent behaviour (Patrick et al, 2015; Bignami et al, 1996; Kavlock et al, 1995), autonomic regulation (Patrick et al, 2015), visual-motor (Moe, 2002), strabismus (Gill et al, 2003), or swallowing (Gewolb et al, 2004) dysfunctions and lower developmental potential (McGlone & Mactier, 2015; Robinson, 2002; Tempel & Espinoza, 1992). Current data cannot clearly differentiate between the long-term neonatal outcomes resulting from the prenatal use of prescription opioids, illicit drugs or opioid maintenance therapy. Buprenorphine and methadone form the mainstay of opioid maintenance therapy during pregnancy. Buprenorphine is considered an attractive alternative, partly due to more favourable neonatal brain growth patterns (Welle-Strand et al, 2009); however, its long-term use cannot be considered benign and has been associated with poor child outcomes to three years of age (Kivisto et al, 2015).

7)The relevance of perturbing apoptosis to gross anatomical malformations

Beginning in the late 1990's the understanding of the mechanism of foetal development began to undergo a significant change through research which identified and defined the concept of

Apoptosis, or programmed cell death (Kerr et al, 1972; Jacobsen et al, 1997; Bartlett, 2018; Mazarakis et al, 1997; Olney et al, 2000). Jacobsen identified the importance of apoptosis in foetal development in 1997. The process itself was described by Kerr et al in 1972 and the Nobel prize for the discoveries was awarded in 2002. It appeared that the foetus developed though the various foetal stages (which have been likened to evolutionary development of the human) by producing an enormous number of pluripotential cells, hugely more than are necessary, which are then selected though signalling from a set of chosen or pre-programmed cells to commit suicide. Research using animal and cell culture models illustrated a developmental plasticity which was controlled by signalling between cellular communities which became ultimately part of organ systems and neurological and other systems through switching off what were deemed to be superfluous or incorrect cells.

The system was controlled by signalling between cells. And since signalling between cells is also the domain of cell receptors and endogenous molecular ligands (for example the neurotransmitter molecules, the small peptide hormones like the endogenous opioids) it was clear that alteration of the developmental landscape through the addition of foreign agents with the ability to alter the sensitivity of the cell communication systems must also carry a serious risk of causing developmental effects, both hidden and morphologically clear in the baby at or after birth.

This problem was quickly addressed through research in the late 1990s and early 2000s since it was clear at that stage that theoretically, the possible causes of major congenital malformations but also hidden neurological, neuropsychiatric and psychosocial effects in children might follow from agents which were not themselves mutagens but which acted by altering the signalling environment during foetal development. It turned out very quickly that almost any agent which affected the homeostatic equilibrium of neurological (and indeed all) development when introduced to the foetus because of the mothers' exposure could cause such effects (Olney et al, 2000). The "teratogenic" effects of alcohol intake and smoking had already been described, though not explained mechanistically.

The opioids operate at the endogenous opioid receptors in the brain, the nervous system and other parts of the organism, as has been pointed out above. The opioid receptors are G-protein coupled receptors. Neural stem cells (cells that develop into the brain and nervous system) are self-renewing and pluripotent cells which give rise to the cells that ultimately make up the brain and nervous system: neurons, astrocytes, oligodendrocytes. In the developing cerebral cortex, neural stem cells differentiate into more committed progenitor cells and migrate into the regions where they lay down the basic structures that finally define the individual. Time lapse images show that cells migrate to the positions where they finally remain in various ways. G-Protein coupled receptors constitute the largest family of transmembrane receptors and are responsible for converting a diverse array of extracellular stimuli into intracellular signalling events. They are involved in a variety of physiological processes such as proliferation, differentiation and migration.

It would therefore have been predictable that the perturbation of the developmental environment by the addition of powerful agents to the extracellular matrix, compounds which have affinity and activity at the G-protein opioid receptors would result in developmental

alterations. And laboratory studies demonstrated such effects (Mizuno et al, 2005). The essential nature of apoptosis in the normal development of palatal fusion is provided by Cuervo (2002). Mid-line fusion defects result from perturbed apoptosis. The importance of apoptosis in the normal development of the heart has been reviewed by van den Hoffa et al (2000). They also reviewed the importance of apoptosis in the effects of teratogens in the production of cardiac malformations.

As pointed out, the role of apoptosis in neural development and disease was described by 1997. This is of interest for the current case since the pharmaceutical companies which were marketing the opioids to pregnant mothers should have realised the potential for serious developmental problems in the foetus which were clear from the reviews which appeared by then (Mazarakis et al, 1997).

However, by the early 1990s it had been already suggested, through a significant body of research, that Cocaine (another G-protein agonist) exposure in utero caused serious alterations in the development of the central nervous system, with major downstream effects implied for the baby and child, including microcephaly and post-natal signs and conditions which were largely the same as those reported for the NAS babies (Nassogne et al, 1995). The question of occult neurophysiological and psychosocial sequelae was clearly implicit.

Evidence that opioids behaved as they were predicted to and caused major birth defects appeared in the results of the National Birth Defect Prevention Study published in 2010 (Broussard et al, 2011; McCarthy, 2015). The study looked at 17,449 cases and 6701 controls. Statistically Significant effects were found for associations between early pregnancy maternal opioid analgesic treatment and certain birth defects, notably heart defects, anencephaly, cleft palate and spina bifida. A list of the most notable birth defects and their Odds Ratios (the ratio between cases and controls) is given in Table 5.

Table 5 Association between maternal opioid analgesic treatment and specific major birth defects in National Birth Defects Prevention Study of 17449 cases and 6701 controls. Significance is starred * in the usual way (Broussard et al, 2011). Odds ratios were adjusted for maternal age, race/ethnicity, education, pre-pregnancy obesity, smoking.

Birth defect	Total no	Odds Ratio (95%CI)
Anencephaly	9	1.7 (0.84-3.4)
Spina bifida**	26	2.0 (1.3-3.2)
Any included heart defect***	211	1.4 (1.1-1.7)
Atrioventricular septal defect*	9	2.4 (1.2-4.8)
Conotruncal defects*	41	1.5 (1.0-2.1)
Tetrology of Fallot*	21	1.7 (1.1-2.8)
Ventricular septal defect*	6	2.7 (1.1-6.3)
L ventricular outflow obstruction*	36	1.5 (1.0-2.2)
Hypoplastic Left heart syndrome**	17	2.4 (1.4-4.1)
R ventricular outflow obstruction*	40	1.6 (1.1-2.3)
Pulmonary valve stenosis**	34	1.7 (1.2-2.6)
Cleft palate	25	1.3 (0.84-2.4)
Hydrocephaly*	11	2.0 (1.0-3.7)

Esophageal atresia	12	1.4 (0.76-2.5)
Gastroschisis*	26	1.8 (1.1-2.9)
Anorectal atresia/ stenosis	18	1.5 (0.9-2.4)
Diaphragmatic hernia	12	1.2 (0.66-2.2)

The study noted that the main results were associated with exposures to Codeine but positive results were also found for Hydrocodone, Oxycodone and Meperidine. Since the mechanism for these effects was by then well described and implicit in the research that had emerged from 1990 onward about neurodevelopment mechanisms, it should have been apparent to those marketing and selling the opioid drugs that warnings should have been given to those prescribing them and to those women taking them. These warnings should have been included in the labelling of the preparations.

8)Communications between the FDA and pharmaceutical companies on opioid teratogenesis.

In connection with this, I have read the following documents:

[redacted]
-JAN-0003-0002176 - Proposed Label Changes for Pregnancy- Tapentadol
-Acquired_Actavis_00184044_-Suboxone/Bupenorphine Labelling
-ACTAVIS0229401 -Actavis Fentanyl Transdermal Patch labelling
[redacted]
INSYS-MDL-000325903_-Morphine Sulphate Provider Insert
PAR_OPIOID_MDL_0000331039-Hydrocodone Ibuprofen Insert

Observations

The responses of the different companies are remarkably consistent, there is very little variation in what has been presented. There are warnings about the consumption of opioids in women who are pregnant or are of child bearing age. The general advice is that the risks to the fetus have to be weighed up against other possible clinical benefits. Nowhere is there the suggestion that any opioid should not be prescribed during pregnancy.

The risk of teratogenesis in the animal studies cited by the manufacturers is largely explained through the mechanism of maternal toxicity rather than a direct teratogenic effect. I have not seen the studies that were relied upon but understand that these studies have not been released under discovery and are not available in the public domain. It is therefore difficult to make an objective assessment of the methodologies applied and the conclusions drawn.

However, it has been argued that if there is no teratognesis at the equivalent of 2x the human dose (on a body surface area basis) or ~ 5 x (on a body weight basis) then it is implied that there is nothing of concern and that opioid medicines should be safe for the medical profession to prescribe to pregnant women. Only gross anatomical malformations seem to

have been considered as toxicological endpoints. Functional deficits, particularly in the nervous system, generally occur at lower doses that those required to produce gross malformations and this has not been alluded to in communications between the FDA and the pharmaceutical companies.

There are a number of criticisms of this approach. Allometric scaling between different species is routine in toxicology. Some of the reasons for needing to do this are:

- Larger animals have lower metabolic rates
- Physiological process of larger animals is slower
- Larger animals required smaller drug dose on weight basis
- Allometry accounts for the difference in physiological time among species
- Allometric scaling is not valid to convert adult doses to fetal or infant doses.

Commonly accepted allometric conversion factors between species are:

Human dose (mg/kg) to mouse dose (mg/kg) - multiply by 12.3

Human dose (mg/kg) to rat dose (mg/kg) - multiply by 7.4

Human dose (mg/kg) to guinea pig dose (mg/kg) - multiply by 4.6

Human dose (mg/kg) to rabbit dose (mg/kg) - multiply by 3.1

Human dose (mg/kg) to dog dose (mg/kg) - multiply by 1.8

For rat and mouse, these allometric scaling ratios of 7.4 and 12.3 respectively, seem to have been ignored in communications to the FDA.

Examples of physiological differences between children and adults that may be significant in terms of toxicological response and which may not scale proportionally or continuously with body weight include the following:

• Respiration rate,

• Glomerular filtration rate,

• Active gastrointestinal absorption of nutrients,

• Composition and activity of intestinal flora

• Percentages of body fat and body water,

• Levels of CYP 450 isoforms and other phase I enzymes,

• Glucuronic acid conjugating ability, Phase 2 enzymes do not reach an efficient level in the infant until about 6 months post-natally.

• Biliary excretion ability, and

• Rates and patterns of growth in particular organs (bones, brain, immune system, etc.) which represent windows of vulnerability for damage during the developmental process.

Regulatory Toxicology

As well as the standard toxicology outlined above, there is an additional consideration that needs to be addressed. The rules of acceptance of a medication under informed consent, though they do apply to the mother, they do not apply to the fetus. In this scenario the fetus is receiving an outside agent, an opioid, which is certainly not being administered for the therapeutic benefit of the fetus. It could therefore be regarded, in this respect, as an external toxic agent.

When considering toxic agents in other life settings, for example pesticide residues on food, regulatory toxicologists try to estimate a 'No Effect Level' (NOEL) from the experimental evidence for a particular toxic agent. This NOEL is then subject to 'Uncertainty Factors' (UFs) which are typically x10 for species difference (when the data comes from a laboratory animal) and a further x10 because of inter-individual variability between humans. Therefore, the NOEL is divided by 10 x 10 = 100 to arrive at Tolerable Daily Intake (TDI). However, in the case of infants and fetuses there is sometimes an additional UF of x 10 applied to account for the additional vulnerability to harm associated with the developmental period. Under this condition the NOEL would have to be divided by 10 x 10 x 10 = 1,000 to arrive at a TDI. An example of this is provided under the US EPA Food Quality Protection Act (FQPA) which applies a UF of 1,000 in the case of infant exposures to toxic xenobiotics in food.

If the experimental data indicate that there is no safe dose then a NOEL cannot be determined and a regulatory TDI cannot be set. Examples of this is are provided by radiation exposure and genotoxic substances.

This brief overview of the regulatory toxicology approach highlights the inadequacy of the safety data put forward by the pharmaceutical industry to the FDA. In my opinion, the risk to the fetus has been understated.

9)Epigenetic effects of opioids

A Mini-Review by Gilardi et al (2018) 'Will Widespread Synthetic Opioid Consumption Induce Epigenetic Consequences in Future Generations?' discusses the animal experimental and human data currently available. Epigenetic changes can alter and regulate the way certain genes express themselves in the absence of mutations. This is achieved by remodelling the structure of the chromatin from 'open' (transcriptionally active) to 'closed' (transcriptionally inactive). A number of molecular mechanisms exist, including DNA methylation and post-translational modification of histones.

The review, while acknowledging the lack of transgenerational studies, "converging evidence suggests that opioids can induce long-lasting transgenerational changes in subsequent generations, particularly concerning drug sensitivity and tolerance, with possible implications for drug abuse vulnerability". A major part of this 'converging evidence' is the animal experimental data that Is available.

It is my opinion that epigenetic transgenerational effects should be considered in any future monitoring program of NAS sufferers

10)Causation – Sir Bradford Hill's methodology

In Bradford Hill's still widely used seminal paper of 1965i, which focuses on how we can move from an observed association to a robust causal inference, he identified nine "features" (often misnamed as "criteria") of the available, and often "ragged", evidence (Vandenbroucke J, Broadbent A & Pearce N, 2015) which, if present, could help justify a robust causal inference. Bradford Hill was careful to point out that even if these features of the evidence (Table 6) were absent, then that did not justify concluding that the agent being evaluated was not causing harm. In other words, the features of the evidence were asymmetrical, a word he did not use despite making the conceptual point very explicit when discussing several of the features of the evidence (Gee, 2008).

Bradford Hill would have approached the evidence with: "the decisive question… whether the frequency of the undesirable event B will be influenced by a change in the environmental feature A?". In this case event B is the diagnosis of NAS and the subsequent negative sequelae. Event A is the exposure of the pregnant mother to opioids.

From Table 6 I conclude that the overall weight of evidence supports a causal link between maternal opioid exposure during pregnancy and the appearance of NAS in the neonate. In my opinion, the causal relationship is strong and is beyond 'more likely than not' i.e. at or around the "balance of probabilities", or the "fair" strength of evidence, which Bradford Hill considered a sufficiency of evidence to justify preventative measures.

Table 6 - The Bradford Hill Approach Applied to NAS

Strength of association: case studies & clinical data indicate clear health impacts in significant proportions of exposed groups
Consistency: The clinical data is consistent with the known action of opioids and is similar across international boundaries.
Specificity: NAS is a syndrome with common neurological symptoms linked to maternal opioid exposure during pregnancy.

Temporality: Opioids have been present throughout recorded human history and therefore it is not possible to establish the position prior to regular human exposure. There is a temporal link in that there has to be maternal opioid exposure prior to the appearance of NAS in the resulting neonate.

Biological gradient: higher maternal opioid exposure often causes greater health effects; but lower dose effects also apparent, suggesting non -linearity

Plausibility: the known effects of opioids through the opioid receptor, which is universally present, support a causal link.

Coherence: animal/human data support a causal link

Experiment: animal experimental data supports a causal relationship between maternal opiate exposure and teratogenic effects (fetal malformations, functional neurological damage) through the principal mechanism of increased apoptosis.

Analogy: Maternal exposure to other teratogenic agents during pregnancy leading to both functional and anatomical teratogenesis; eg lead, mercury, di-ethyl stilbestrol, thalidomide.

11)Summary and opinion

Opioids all act in common through the opioid receptor system, which is universally present in vertebrate life forms and is well conserved throughout their evolutionary history. Therefore, this class of drugs has a common mode of action.

Opioid pharmaceutical agents affect the rate of apoptosis in development. The rate of apoptosis (programmed cell death) is critical for the normal development of the fetus and perturbing that rate will lead to teratogenesis, both morphological and functional. Therefore, there is a recognised pathological mechanism in common across this class of pharmaceutical agents.

There are no questions concerning foetal opioid dose to address. The Plaintiffs in this Class Action all had significant exposure to pre-natal opioid pharmaceuticals via their mothers. This was at a level which subsequently led to the postpartum diagnosis of NAS. This diagnosis is casually linked with a higher risk of suffering a congenital malformation. It also causally linked with a higher risk of neurological damage which could be expressed through various latent negative health impacts. These are the reasonably certain consequences of their

exposures, which are the result of subcellular or other physiological changes and can also be manifested in physical or mental injury or disease.

Prescribing opioids to women during pregnancy will lead to fetal damage. This is because of the known toxicity of opioids to the fetus which leads to increased risk of latent disease in the child post-natally. This should be made clear to women of child bearing age who are on opioid maintenance therapy and who are at risk of becoming pregnant. In my opinion, regimes for maternal opioid withdrawal should be considered as a primary part of any risk benefit considerations. The manufacturer's submissions to the FDA do not indicate any support for this approach which, in my opinion, did not reflect known risks of neurologic, developmental or teratogenic effects.

The establishment of Scientific Panels to participate in a medical monitoring program would, in my opinion, be beneficial for the following reasons. Monitoring for the post-natal consequences NAS is reasonable and necessary, according to contemporary scientific principles. The monitoring program should include periodic diagnostic medical examinations as there is clinical value in early detection and diagnosis. Therefore, this is different from a typical post-natal monitoring regime in the absence of exposure. The collection of prospective epidemiological data from a large cohort of NAS sufferers will lead to very robust studies that will deliver a medical benefit to a wider group of NAS sufferers than just those who have received prescription opioids during pregnancy. There will be beneficial read-across data that is relevant to other classes of opioid exposed fetuses. Such Scientific Panels should be composed of experts from the multiple medical and scientific disciplines that are required to fully understand this complex condition, including (but not necessarily restricted to): paediatricians, epidemiologists, physicians specialising in opioid addiction, psychiatrists, behavioural psychologists, toxico-pathologists, neurobiologists.

In my opinion, on the balance of scientific and medical probabilities, these negative outcomes are attributable to the treatment of the mothers with opioids. This includes treatment of mothers-to-be prior to pregnancy, when addictions can be established, and then during pregnancy at a dose adequate to induce NAS in their infant, via the common mode of action of this class of medicines and through the common pathological mechanism identified.

12)Statement of Truth

I confirm that I have made clear which facts and matters referred to in this report are within my own knowledge and which are not. Those that are within my own knowledge I confirm to be true. The opinions I have expressed represent my true and complete professional opinions on the matters to which they refer.

Dr. C.V. Howard 02/12/2019

References

Anand KJS & Campbell-Yeo M. Consequences of prenatal opioid use for newborns. Acta Pædiatrica. 2015 104: 1066–1069

Bains R.K., Sibbons P.D., Murray R.D., Howard C.V. van Velzen D. (1996).'Stereological estimation of the absolute number of glomeruli in the kidneys of the lamb.' Research in Veterinary Science, 60:122-125.

Barker DJ, Winter PD, Osmond C, Margetts B, Simmonds SJ. Weight in infancy and death from ischaemic heart disease. Lancet. 1989; 2:577–80.

Bartlett Z (2018) Apoptosis in embryonic development. The Embryo Project Encycopedia. https://embryo.asu.edu

Bignami G (1996) Economical test methods for developmental neurobehavioural toxicity.

Environmental Health Perspectives 104(S2) 285-298

Bradford Hill A. The Environment And Disease: Association Or Causation? In: Proceedings of the Royal Society of Medicine. Vol 58. Royal Society of Medicine Press; 1965:295-300.

Broussard C, Rasmussen SA, Reefhuis J et al (2011) Maternal treatment with opioid analgesics and risk for birth defects. Am.J.Obst.Gynecol. 204: 314 e1-11.

Changeux JP, Danchin A (1976). Selective stabilisation of developing synapses as a mechanism for the specification of neuronal networks. Nature 264: 705–712.

Conradt E, Sheinkopf SJ, Lester BM, Tronick E, LaGasse LL, Shankaran S, et al. Prenatal substance exposure: neurobiologic organization at 1 month. J Pediatr 2013; 163: 989–94 e1.

Coyle MG, Salisbury AL, Lester BM, Jones HE, Lin H, GrafRohrmeister K, et al. Neonatal neurobehavior effects following buprenorphine versus methadone exposure. Addiction 2012; 107 (Suppl. 1): 63–73.

Creeley CE, Dikranian KT, Johnson SA, Farber NB, Olnesy JW (2013) Alcohol induced apoptosis of oligodendrocytes in the fetal macaque brain. Acta Neuropathologica Communications 1: 23

Cuervo R, Concepcio´n Valencia, Roshantha A. S. Chandraratna, and Luis Covarrubias. Programmed Cell Death Is Required for Palate Shelf Fusion and Is Regulated by Retinoic Acid. Developmental Biology 245, 145–156 (2002).

Desai RJ, Huybrechts KF, Hernandez-Diaz S, Mogun H, Patorno E, Kaltenbach K, et al. Exposure to prescription opioid analgesics in utero and risk of neonatal abstinence syndrome: population based cohort study. BMJ 2015; 350: h2102.

Fodor A, Timar J, Zelena D. Behavioral effects of perinatal opioid exposure. Life Sci 2014; 104: 1–8.

Gee D. Establishing evidence for early action: the prevention of reproductive and developmental harm. Basic Clin Pharmacol Toxicol. 2008;102(2):257–266.

Gewolb IH, Fishman D, Qureshi MA, Vice FL. Coordination of suck-swallow-respiration in infants born to mothers with drug-abuse problems. Dev Med Child Neurol 2004; 46: 700–5.

Gilardi F, Marc Augsburger and Aurelien Thomas. Will Widespread Synthetic OpioidConsumption Induce Epigenetic Consequences in Future Generations? Front. Pharmacol. 2018, 9:702.. doi: 10.3389/fphar.2018.00702

Gill AC, Oei J, Lewis NL, Younan N, Kennedy I, Lui K. Strabismus in infants of opiate-dependent mothers. Acta Paediatr 2003; 92: 379–85.

Gutstein H B and Akil H (2006) Opioid Analgesics. Ch 26 In- Brunton LL, Lazo JS, Parker KL Goodman and Gilman's The pharmacological basis of Therapeutics. New York: McGraw Hill.

https://webbook.nist.gov/cgi/cbook/ Molecular structures and other characteristics of individual opioids: NIST Chemistry Web Book.

Harlan, R. E. & Song, D. D. Prenatal morphine treatment and the development of the striatum. Regul. Pept. 54, 117–118 (1994)

Hinchliffe S.A., Lynch M.R.J., Sargent P.H., Howard C.V., van Velzen D. (1992). 'The effect of human intrauterine growth retardation on the development of renal nephrons.' British Journal of Obstetrics and Gynaecology 99: 296-301.

Hu, S., Sheng, W. S., Lokensgard, J. R. & Peterson, P. K. Morphine induces apoptosis of human microglia and neurons. Neuropharmacology 42, 829–836 (2002)

Jacobsen MD, Weil M, Raff (1997) Programmed cell death in animal development. Cell 88 347-54

Kavlock RJ, Woodrow Seltzer R (1995) The road to embryologically based dose-response models. Environmental Health Perspectives. 104 S1 107-121

Kerr JF, Wyllie AH, Currie A R (1972) Apoptosis: A basic biological phenomenon with wide ranging implications in tissue kinetics. British Journal of Cancer. 26: 239-57

Kivisto K, Tupola S, Kivitie-Kallio S. Prenatally buprenorphine-exposed children: health to 3 years of age. Eur J Pediatr 2015. doi:10.1007/s00431-015-2562-0 [Epub ahead of print].

Liu AJW, Sithamparanathan S, Jones MP, et al Growth restriction in pregnancies of opioid-dependent mothers Archives of Disease in Childhood - Fetal and Neonatal Edition 2010;95:F258-F262.

Lu R, Liu X, Long H, Ma L. Effects of prenatal cocaine and heroin exposure on neuronal dendrite morphogenesis and spatial recognition memory in mice. Neurosci Lett 2012; 522: 128–33.

McCarthy M (2015) Opioids are commonly prescribed to women of reproductive age despite birth defect risk, US CDC study shows. BMJ 2015 350 h456

McGlone L, Mactier H. Infants of opioid-dependent mothers: neurodevelopment at six months. Early Hum Dev 2015;91:19–21.

Mazarakis ND, Edwards AD, Mehmet H (1997) Apoptosis in neural development and disease. Archives of diseases in childhood. 77 F165-F170

Mizuno N, Kokubu H, Sato M et al. (2005) G-Protein coupled receptor signalling through Gq and JNK negatively regulates neural progenitor cell migration. PNAS 102 (35) 12365-12370

Moe V. Foster-placed and adopted children exposed in utero to opiates and other substances: prediction and outcome at four and a half years. J Dev Behav Pediatr 2002; 23: 330–9.

Nassogne M-C, Evrard P, Courtoy PJ (1995) Selective neuronal toxicity of Cocaine in empryonic mouse brain co-cultures. PNAS 92 11029-11033

Oates JA (2006) The science of drug therapy. Ch 5 In- Brunton LL, Lazo JS, Parker KL Goodman and Gilman's The pharmacological basis of Therapeutics. New York: McGraw Hill.

O'Brien P (2006) Drug addiction and drug abuse. Ch 23 In- Brunton LL, Lazo JS, Parker KL Goodman and Gilman's The pharmacological basis of Therapeutics. New York: McGraw Hill.

Olney PD, Farber NB, Wozniak DF, Jevtovik-Todorovi V, Ikonomidou C (2000) Environmental agents that have the potential to trigger massive apoptopic neurodegeneration in the developing brain. Environmental Health Perspectives 108 S3 383-388

Patrick S, Dudley J, Martin PR, Harrel FE, Warren MD, Hartmann KE, Wesley Ely KE, Grijalva CG, Cooper WO (2015) Prescription opioid epidemic and infant outcomes. Pediatrics 135(5) 842-850

Reed MG, Howard CV & Staats de Yanes G (2010). One-Stop Stereology: the estimation of 3D
parameters using Isotropic Rulers. Journal of Microscopy, 239: 54-65.

Robinson SE. Effects of perinatal buprenorphine and methadone exposures on striatal cholinergic ontogeny. Neurotoxicol Teratol 2002; 24: 137–42.

Sadraie SH, Kaka GR, Sahraei H, Dashtnavard H, Bahadoran H, Mofid M, et al. Effects of maternal oral administration of morphine sulfate on developing rat fetal cerebrum: a morphometrical evaluation. Brain Res 2008; 1245: 36–40.

Stein C (2016) Opioid Receptors. Ann Rev.Med. 67: 433-51

Stephens CW (2011). The evolution of vertebrate opioid receptors. Front Biosci. 14:1247-1269

Svensson A-L, Bucht N, Hallberg M, Nyberg F (2008) Reversal of opiate induced apoptosis by human recombinant growth hormone in murine foetus primary hippocampal neuronal cell cultures. PNAS 105 (20) 7304-7308

Tau GZ and Peterson BS (2010). Normal Development of Brain Circuits. Neuropsychopharmacology Reviews 35: 147-168

Tempel A, Espinoza K. Morphine-induced downregulation of mu-opioid receptors and peptide synthesis in neonatal rat brain. Ann N Y Acad Sci 1992; 654: 529–30.

Vandenbroucke J, Broadbent A, Pearce N. Causality and causal inference in epidemiology - the need for a pluralistic approach. Int J Epidemiol. 2015:1-12.

Vestal-Laborde AA, Eschenroeder AC, Bigbee JW, Robinson SE, Sato-Bigbee C. The opioid system and brain development: effects of methadone on the oligodendrocyte lineage and the early stages of myelination. Dev Neurosci 2014; 36: 409–21.

Walhovd KB, Moe V, Slinning K, Due-Tonnessen P, Bjornerud A, Dale AM, et al. Volumetric cerebral characteristics of children exposed to opiates and other substances in utero. NeuroImage 2007; 36: 1331–44.

van den Hoffa M. J.B.,, Stefan M. van den Eijnde, Szabolcs Vira´gh, Antoon F.M. Moorman. Programmed cell death in the developing heart. Cardiovascular Research 45 (2000) 603–620

Welle-Strand GK, Skurtveit S, Jones HE, Waal H, Bakstad B, Bjarko L, et al. Neonatal outcomes following in utero exposure to methadone or buprenorphine: a National Cohort Study of opioid-agonist treatment of Pregnant Women in Norway from 1996 to 2009. Drug Alcohol Depend 2013; 127: 200–6.

Wolff K, Perez Montenajo R (2014) Opioid Neonatal abstinence syndrome: Controversies and implication for practice. Current drug abuse reviews. 7 44-58

Yanai, J. et al. Functional changes after prenatal opiate exposure related to opiate receptors' regulated alterations in cholinergic innervation. Int. J. Neuropsychopharmacol. 6, 253–265 (2003)

Yazdy MM, Desai RJ and Brogly SB (2015) Prescription opioids in pregnancy and birth outcomes a review of the literature. J.Pediatric Genetics. 4 (2) 56-70

Yuan Q, Rubic M, Seah J, Rae C, Wright IM, Kaltenbach K, et al. Do maternal opioids reduce neonatal regional brain volumes? A pilot study J Perinatol 2014; 34: 909–13.

Zandi-Nejad K1, Luyckx VA, Brenner BM. Adult hypertension and kidney disease: the role of fetal programming Hypertension. 2006 Mar;47(3):502-8. Epub 2006 Jan 16.